(Page 5, Paragraph 6, line 32, please insert SEQ. ID. NO. 2:)

Figure 3 shows a product-ion spectrum of the $[M + {}^{107}Ag]^+$ ion of GGEGG (SEQ. ID. NO. 2) at an E_{cm} of 2.0 eV.

(Page 5, Paragraph 7, line 34, please insert SEQ. ID. NO. 3:)

Figure 4 shows the composite product-ion spectrum of the $[M + {}^{109}Ag]^+$ ion of bradykinin, RPPGFSPFR (SEQ. ID. NO. 3), at E_{cm}s of 2.0 and 2.5 eV.

B1

Page 6, Paragraph 1, line 2, please insert SEQ. ID. NO. 4:

B2

Figure 5 shows a product-ion spectrum of the $[M + {}^{107}Ag]^+$ ion of dynorphin A fragment 1-7, YGGFLRR (SEQ. ID. NO. 4), at E_{cm} = 1.9 eV.

Page 11, Paragraph 1, line 6, please insert SEQ. ID. NO. 1:

B3

Figure 1 shows that the product-ion spectra of the $[M + {}^{107}Ag]^+$ ion (silver has two stable isotopes, ^{107}Ag and ^{109}Ag, of almost equal abundance; the product-ion spectra shown in this article will be those of either one of the two isotopes) of leucine enkephalin, YGGFL (SEQ. ID. NO. 1), collected under different E_{cm}s: (a) 1.5, (b) 2.0, and (c) 2.5 eV; (d) is the composite of (a), (b), and (c). It is well-known that individual product-ion yield is strongly dependent on collision energy (Dawson et al. (*Org. Mass Spectrom.* 17, 205-211 (1982); Dawson et ala. (*Org. Mass Spectrom.* 212-217 (1982)); a necessary step in sequencing is to acquire product-ion spectra under several collision energies to find the best spectra for sequencing. Summing the spectra to produce a composite provides

a convenient way of presenting a minimal number of searchable mass spectra to the triplet/doublet identification algorithm. An alternative method to generate a wide range of searchable product ions is to acquire a product-ion spectrum with *m/z*-dependent E_{cm} function. Figure 2 shows such a product-ion spectrum also for the $[M + {}^{107}Ag]^+$ ion of leucine enkephalin. It was acquired with a linear E_{cm} function from 2.5 eV for *m/z* = 30 to 1.5 eV for *m/z* = 663, but otherwise under the same experimental conditions as those in Figure 1 (the scan time equalled the total scan time of the three individual E_{cm}s). It is readily apparent that the spectra are not identical (due to differences in exact collision energies), but the overall spectral quality of the two is similar.

B3

Page 13, line 2, please insert SEQ. ID. NO. 1 and SEQ. ID. NO. 5:

ion of tyrosine (theoretical $\Delta m/z$ = 136.2). Thus, the determined amino acid sequence of leucine enkephalin is Y-G-G-F-L/I (SEQ. ID. NO. 1 /SEQ. ID. NO. 5) and (SEQ. ID. NO. 5), which is correct.

Page 13, Paragraph 1, line 5 and 22, please insert SEQ. ID. NO. 2:

EXAMPLE 2

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Figure 3 shows a product-ion spectrum of the $[M + {}^{107}Ag]^+$ ion of GGEGG (SEQ. ID. NO. 2), glycylglycylglutamylglycylglycine. Sequencing of the argentinated peptide is straightforward and similar to that discussed in Example 1 for leucine enkephalin. The difference in *m/z* values between the $[M + Ag]^+$ ion and the first $[b_n + OH + Ag]^+$ ion is 482.1 - 425.1 = 57.0, which

identifies the C-terminal residue as glycine (theoretical $\Delta m/z$ = 57.0). The difference in m/z values between the first $[b_n + OH + Ag]^+$ and the second $[b_n + OH + Ag]^+$ is 425.1-368.0 = 57.1; this identifies the residue preceding the C-terminus as, again, glycine. Repeating the procedure yields the next residue, glutamic acid (experimental $\Delta m/z$ = 128.9 versus theoretical $\Delta m/z$ = 129.0). The triplet peak pattern disappears beyond glutamic acid; however, manual interpretation reveals further sequence information. The $[a_1 - H + Ag]^+$ in this product-ion spectrum is weak. However, the small m/z value of the $[a_n - H + Ag]^+$ ion of the last triplet, 193.1, strongly suggests that it is the $[a_2 - H + Ag]^+$ ion. This makes G-G as the only possible option for the N-terminal and second residues (theoretical m/z = 193.0). Furthermore, Figure 3 shows a small peak at m/z = 136.0, which is assignable as the $[a_1 - H + Ag]^+$ ion (theoretical m/z = 136.0, assuming the first residue is glycine). Thus, the determined sequence is G-G-E-G-G (SEQ. ID. NO. 2).

Page 13, Paragraph 2, line 25, please insert SEQ. ID. NO. 3:

Page 14, line 3, please insert SEQ. ID. NO. 3:

EXAMPLE 3

The following example relates to a longer peptide bradykinin. A product-ion spectrum of the $[M + {}^{109}Ag]^+$ ion of bradykinin, RPPGFSPFR (SEQ. ID. NO. 3), is shown in Figure 4. Sequencing of the $[M + H]^+$ ion of bradykinin is considered difficult because the external proton is believed to be sequestered by the highly basic guanidine groups on the side chains of the two arginine

residues, thus rendering it unavailable for binding to the amidic functional groups and inducing charge-proximal fragmentation along the peptide backbone (Alexander et al. (1990); Tang et al. (1993); Burlet et al. (1992); Cox et al. (1996); Summerfield et al. (1997)). The Ag + ion, however, appears to bind to many different sites on the peptide, as evident from the relative richness of the fragmentation pattern in Figure 4. Table 1 summarizes the triplets found and the residues identified using the above approach. It is apparent that only a partial sequence of five residues starting from the C-terminal end of the peptide has been solved - FSPFR (SEQ. ID. NO. 3); the major advantage of sequencing argentinated peptides relative to protonated peptides is the triplet relationship which greatly facilitates product-ion assignment. Bradykinin has the highly basic arginine as its C-terminal residue, which also binds strongly to the silver ion (Lee at al. *J. Am. Soc. Mass Spectrom.* (1998)). In fact, it is our observation that peptides that have C-terminal methionine, lysine, and arginine residues tend to yield relatively strong $[y_n + H + Ag]^+$ product ions (Li et al. (1997); Chu et al. (not published)). In the search algorithm, presence of the corresponding $[y_n + H + Ag]^+$ ion is used as confirmation of the cleaved residue. For bradykinin, the $[y_n + H + Ag]^+$ ions for n = 3-5 have been observed (Figure 4 and Table 1), which confirms results of the triplet search. In Figure 4, the triplet signal corresponding to cleavage of the proline residue is weak; this is actually a confirmation of the proline-residue assignment. Proline is the only residue from which an oxazolone $[b_n - H + Ag]^+$ cannot be formed and of which the relatively weak $[b_n - H + Ag]^+$ ion is believed to be a ketene (Lee et al., *J. Am. Chem. Soc.*

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(1998)). The assignment of proline can often be confirmed by the presence of the appropriate $[y_n + H + Ag]^+$ ions or the identification of the next triplet; the difference in the *m/z* values of the $[b_n + OH + Ag]^+$ ions for $n = 1$ and $n = 3$ is 1010.3 - 766.5 = 243.8, which can only mean a combination of cleavage of phenylalanine and proline.

Page 14, Paragraph 1, line 26, please insert SEQ. ID. NO. 4:

EXAMPLE 4

Figure 5 shows a product-ion spectrum of the $[M + {}^{107}Ag]^+$ ion of dynorphin A fragment 1-7, YGGFLRR (SEQ. ID. NO. 4), another highly basic peptide. The triplets and the residues identified using the search algorithm are tabulated in Table 2. Again, the partial sequence determined, - -FLRR, is confirmed by the presence of the appropriate $[y_n + H + Ag]^+$ ions.

Page 19, peptide 1, determined sequence, please insert SEQ. ID. NO. 6:

Page 19, peptide 1, actual sequence, please insert SEQ. ID. NO. 7:

Page 19, peptide 2, determined sequence, please insert SEQ. ID. NO. 8:

Page 19, peptide 2, actual sequence, please insert SEQ. ID. NO. 9:

Page 19, peptide 3, determined sequence, please insert SEQ. ID. NO. 10:

Page 19, peptide 3, actual sequence, please insert SEQ. ID. NO. 11:

Page 19, peptide 4, determined sequence, please insert SEQ. ID. NO. 12:

Page 19, peptide 4, actual sequence, please insert SEQ. ID. NO. 13:

Page 19, peptide 5, determined sequence, please insert SEQ. ID. NO. 14:

Page 19, peptide 5, actual sequence, please insert SEQ. ID. NO. 15:

Table 3

Sequencing of Tryptic Peptides

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peptide	determined sequence	actual sequence
1	-FAGK (SEQ. ID. NO. 6)	LIFAGK (SEQ. ID. NO. 7)
2	-(L/I)FVK (SEQ. ID. NO. 8)	MQIFVK (SEQ. ID. NO. 9)
3	-TGK (SEQ. ID. NO. 10)	TLTGK (SEQ. ID. NO. 11)
4	-DVEK (SEQ. ID. NO. 12)	GDVEK (SEQ. ID. NO. 13)
5	-V(Q/K)K_a (SEQ. ID. NO. 14)	IFVQK (SEQ. ID. NO. 15)

[a] K and Q are isobaric; however, since trypsin cleaves only on the C-terminal side of K and R, the identity of the C-terminal residue is unambiguous, but that of preceding residues is not.

Page 20, paragraph 2:

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MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQR**LIFAGK**QLE DGRTLSDYNIQKESTLHLVLRLRGG (SEQ. ID. NO. 16)